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Populations of some molds in water-damaged homes may differ if the home was constructed with gypsum drywall compared to plaster

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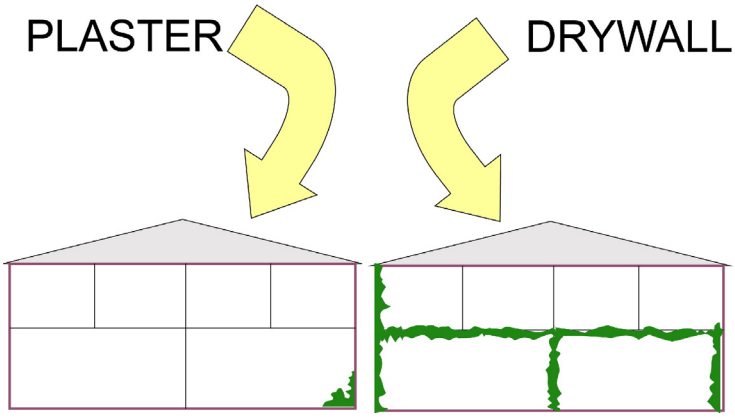
HIGHLIGHTS

- Mold exposure has been associated with asthma symptoms for many years.
- Gypsum drywall replaced plaster in U.S. home construction after World War II.
- Mold populations in the water-damaged homes changed with drywall's introduction.

GRAPHICAL ABSTRACT



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ABSTRACT

Starting in the 1940s, gypsum drywall began replacing plaster and lathe in the U.S. home construction industry. Our goal was to evaluate whether some mold populations differ in water- damaged homes primarily constructed with gypsum drywall compared to plaster. The dust samples from the 2006 Department of Housing and Urban Development's (HUD) American Health Homes Survey (AHHS) were the subject of this analysis. The concentrations of the 36 Environmental Relative Moldiness Index (ERMI) molds were compared in homes of different ages. The homes ($n = 301$) were built between 1878 and 2005. Homes with ERMI values >5 ($n = 126$) were defined as water-damaged. Homes with ERMI values >5 were divided in the years 1976 to 1977 into two groups, i.e., older ($n = 61$) and newer ($n = 65$). Newer water-damaged homes had significantly ($p = 0.002$) higher mean ERMI values than older water-damaged homes, 11.18 and 8.86, respectively. The Group 1 molds *Aspergillus flavus*, *Ammophilus fumigatus*, *Aspergillus ochraceus*, *Cladosporium sphaerospermum* and *Trichoderma viride* were found in significantly higher concentrations in newer compared to older high-ERMI homes. Some mold populations in water-damaged homes may have changed after the introduction of gypsum drywall.

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1. Introduction

The Department of Housing and Urban Development (HUD) periodically conducts national surveys of the United States (U.S.) housing

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stock to evaluate a wide range of conditions in U.S. homes. In the HUD 2006 American Healthy Homes Survey (AHHS), mold populations in the U.S. housing stock were quantified. Settled-dust samples were collected from a representative sample of U.S. homes ($n = 1083$) and the population of 36 molds commonly found in homes were quantified by mold specific quantitative PCR (MSQPCR) analysis (Vesper et al. 2007). These results were used to create a scale of mold contamination called the Environmental Relative Moldiness Index (ERMI) (Vesper et al. 2007). This scale ranges from about −10 to 30, i.e., lowest to highest mold contamination. The ERMI scale has now been applied in six epidemiological studies of asthma in the U.S. (Vesper and Wymer, 2016).

In one example study, a ten-year prospective study in Cincinnati Ohio, Reponen et al. (2011) found that infants living in homes with high ERMI values (≥5.2) were more than twice as likely to develop asthma by age seven. They also found that the ERMI values in these children's homes were significantly correlated with the age of the home, i.e., older homes (built before 1955) had higher average ERMI values than newer homes (Reponen et al. 2013). Three of the 36 ERMI-molds, *Aspergillus ochraceus*, *Aspergillus unguis* and *Penicillium variabile*, were found in significantly higher concentrations in the homes of infants that developed physician diagnosed asthma at age seven (Reponen et al. 2012). The other five epidemiological studies also revealed a positive relationship between homes with high ERMI values and occupant asthma (Vesper and Wymer, 2016). If homes with high ERMI values are associated with asthma, we wondered if the mold populations in U.S. homes might have increased in a manner consistent with the increase in asthma prevalence in the U.S.

The prevalence of asthma in the U.S. has doubled since the 1970's (CDC, 2014). Although we know there is a genetic predisposition for asthma (Ortega and Meyers, 2014), changes in the genetic make-up of the U.S. population cannot account for the timing and relatively rapid increase in asthma prevalence in the second half of the 20th century (Johnson et al., 2002). Of course, many factors and exposures have changed in the second half of the 20th century. For our analysis, the focus was limited to determining whether the populations of ERMI-molds are different in water-damaged homes with plaster and lathe construction compared to water-damaged homes containing gypsum drywall (also known as drywall, gypsum board, or sheetrock).

2. Methods

2.1 Study samples

The selection of homes and the collection of samples and questionnaire data for the HUD AHHS was described previously (HUD, 2015). The AHHS was reviewed for human subject involvement by the Westat Institutional Review Board and a Confidentiality Certificate protecting the identity of the survey respondents issued by the National Institute of Environmental Health Sciences.

2.2 Dust sample collection

The dust samples for the AHHS samples ($n = 1083$) were collected in 2006. Settled floor dust samples were collected by vacuuming a 2 m^2 area for five minutes in the living room and then the bedroom with a MiTest™ sampler (Indoor Biotechnologies, Charlottesville, VA). The dust was returned to the laboratory and then sieved through a 300 μ pore size nylon mesh (Gilson Company, Inc., Lewis Center, OH) and shipped frozen on ice packs overnight to the analytical laboratory and maintained frozen at −20 °C until analyzed.

2.3 Mold analysis

For the quantification of the molds, five mg of each sieved-dust sample was extracted and the DNA purified using the DNA-EZ kit (GeneRite, Monmouth Junction, NJ) (Vesper et al., 2015b). Each of the 36 ERMI molds was quantified by mold specific quantitative PCR (MSQPCR) assays, as previously described (Haugland and Vesper, 2002; Haugland et al., 2004). Briefly, the standard MSQPCR assays contained 12.5 μl of "Universal Master Mix" (Applied Biosystems Inc., Foster City, CA), 1 μl of a mixture of forward and reverse primers at 25 μM each, 2.5 μl of a 400 nM TaqMan probe (Applied Biosystems Inc.), 2.5 μl of 2 mg/ml fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO) and 2.5 μl of DNA free water (Cepheid, Sunnyvale, CA). To this mix was added 5 μl of the DNA extract from the sample. All primer and probe sequences used in the assays are at the website: https://irp-cdn.multiscreensite.com/c4e267ab/files/uploaded/gCQnkBNWQuSD96fPIikY_EPA_Technology%20for%20Mold%20Identification%20and%20Enumeration.pdf.

The mold contamination or ERMI value in each home was then calculated, as described below (Vesper et al., 2007).

2.4 Calculating the Environmental Relative Moldiness Index (ERMI) value

The calculation of the ERMI value for a home is based on the MSQPCR analysis of 36 molds classified into two groups. Group 1 molds include 26 species related to water damage while Group 2 species are commonly found in homes across the United States, even without water damage, and come primarily from outdoors. The ERMI calculation takes the results from the concentrations (cells/mg dust) of each of 36 molds and mathematically converts these into a single number, as shown in Eq. (1).

$$\mathrm{ERMI} = \sum_{i=1}^{26} \log_{10}(s_{1i}) - \sum_{j=1}^{10} \log_{10}(s_{2j}) \quad (1)$$

The concentration of each of the 26 Group 1 molds is converted to a log and then the "Sum of the Logs of the Group 1" (SLG1) molds is determined (s_1). Similarly, the concentration of each of the 10 Group 2 molds are converted to a log and then the "Sum of the Logs of the Group 2" (SLG2) molds is determined (s_2). The arithmetic difference (SLG1 minus SLG2) is the ERMI value for the home (Vesper et al., 2007).

The ERMI values for the AHHS homes were assembled from lowest to highest to create the ERMI scale of mold contamination. This scale ranges from about −10 to 30, i.e., lowest to highest mold contamination. Homes with an ERMI values above 5 are in the highest mold contamination quartile of homes in the U.S. (Vesper et al., 2007).

2.5 Statistical analysis

The correlation between ERMI values and the age of the AHHS homes was based on the Spearman rho correlation test. The statistical analysis of the differences in concentrations in the dust samples of individual mold species between AHHS homes built before 1976 and after 1977 compared was evaluated by the Student *t*-test. Analyses were performed in SAS version 9.3 (SAS Institute, Cary NC) and R version 2.14 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

The data for the year the home was built was available from homeowner questionnaire responses for 301 of the AHHS homes. These homes were built between 1878 and 2005 and the ERMI values for these homes ranged from −9.53 to 27.02. There was no correlation between when the homes were built and the ERMI values for the homes (Spearman rho −0.002; $p = 0.96$) (data not shown). However, if only the homes with ERMI values >5 ($n = 126$) were considered, there was a positive, significant (Spearman 0.17; $p = 0.04$) correlation between the year the home was built and the ERMI value, i.e., higher ERMI values were found in the newer, water-damaged homes compared to older, water-damaged homes (Fig. 1A and 1B).